HSV 1 (gG1) ELISA
(recombinant)
HSV 2 (gG2) ELISA
(affinity-purified)
IgG / IgM Testkit

Order No.:

HSV 1 (gG1)	**EC130.00**
HSV 2 (gG2)	**EC131.00**

Color Coding:

HSV 1 (gG1)	**red/black**
HSV 2 (gG2)	**red/dark blue**

FOR IN VITRO DIAGNOSIS ONLY

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1. Intended Use

The HSV 1 (gG1) – resp. the HSV 2 (gG2) ELISA is intended for the semiquantitative and qualitative detection of specific IgG/IgM -antibodies against *Herpes simplex Virus* (HSV) type 1 resp. type 2 in human serum.
The serology is suitable for the detection of the immune status and as Herpes exclusion. The use of the type-specific glycoproteins G, gG1 resp. gG2 enables the differentiation between HSV 1 and HSV 2 to determine the seroprevalence, to identify potential virus-carrier and for the risk calculation and prevention of the Herpes neonatorum.
The IgM result must not be observed isolated from the IgG result.
The diagnosis of the genital-herpes must be confirmed with the pathogen detection.
The serology is not suitable for the detection of new-born Herpes, as the immune system of a baby is not completely developed at the date of its birth. However it can be used in retrospect to measure the transplacentally transferred anti-HSV-2 IgG-antibodies.

2. Diagnostic Relevance

Herpes simplex viruses are widely spread throughout the population. The transmitted results from direct contact with infected secretions from either a symptomatic or an asymptomatic host. Therefore the contamination starts already in the early child-age. However these primary infections remain asymptomatical in over 90% of the cases, a latent infection is established in the regional ganglia as a rule. For the understanding of the pathogenesis of HSV-infection the fact that latent persistent viruses in the ganglia cells may be reactivated is of important meaning. The further spreading of the virus is favourabled by the asymptomatical virus expression throughout saliva and genital secretion. In the orafacial area the HSV1-infections prevail, whereas in the genital area the infections are mostly caused by HSV 2. Only a small part (5-30%) is generated by HSV 1 (11, 14).
One of the most serious consequences of genital herpes is neonatal herpes (2). Without therapy, mortality for untreated infants who develop disseminated infection exceeds 70% with half of the survivors developing neurological impairment (14). Almost all neonate HSV 2 infections are acquired by passage through an infected birth canal (7). Most mothers (60-80%) who transmit HSV to their children are asymptomatic at delivery (14). Transmission rates are much higher when the mother is experiencing a primary or initial genital infection (50%) (14) versus a recurrent infection (<5%) (4, 5, 9). CDC recommends that *„...prevention of neonatal herpes should emphasize the prevention of acquisition of genital HSV infection during late pregnancy. Susceptible women whose partners have oral or genital HSV infection, or those whose sex partners infection status is unknown, should be counseled to avoid unprotected genital and oral sexual contact during late pregnancy“* (7).

Viral isolation, direct fluorescent antibody (DFA) testing, and serology can be used to diagnose HSV infections. Disadvantages of the first two methods are however, length of culture time, specimen collection and transport difficulties, procedural complexity, and other variables that are associated with DFA and culture (1, 7). However, due to the significant cross-reactivity between HSV 1 and HSV 2, the serological assays, that use virus lysates as antigens, are not sufficiently suited to differentiate HSV 1 infections from HSV 2 infections. Due to the high contamination with HSV 1, the serological status for HSV 2 can be detected hardly reliable with such methods (14).
Intrathecal IgG-antibodies occur only 8 – 10 days after the clinical symptoms in a present Herpes encephalitis. IgM antibodies are not regularly developed, but if so, it is in short term appearance and in very low concentration. This means the serology can be used as a confirmatory tool of the clinical diagnosis retroactively.

The genital HSV 1-infections recurrent considerably more rarely than HSV 2-infections. A previous infection with genital HSV1 seems to give a certain protection of infections with HSV 2 respectively allays the symptoms or entirely prevent them(10). A previous oral HSV 1 infection does not protect against a genital HSV 2 infection (14). The clinical picture of genital herpes corresponds those of other ulceration of the sexual organs and has therefore to be differentiated against *Haemophilus durcreyi, Treponema pallidum* and *Chlamydia trachomatis* (7).

Herpes simplex CSF diagnosis
In contrast to the serological diagnosis of HSV infections, what is most important in CSF diagnosis is the reliable detection of endogenous synthesis of pathogen-specific antibodies in the CNS, rather than any differentiation between the pathogen species HSV-1 and HSV-2. As a result of the combination of highly purified HSV-1 and HSV-2 lysate antigens in the VT HSV

screening test, this test system provides a very suitable screening test for the CSF diagnosis of HSV infections of the CNS, as it is highly sensitive. A broad spectrum of highly purified HSV antigens is used in the VT-HSV screen. This not only leads to the desired high sensitivity, but also to the equally desirable specificity with respect to differentiation from CNS infections with other neutrotropic pathogens of the herpes virus group.

We therefore recommend that the antibody index (AI) should initially be determined in Herpes simplex diagnostic testing with the HSV Screen ELISA.

If there is the additional aim of achieving differentiation between HSV1 and HSV2 after detection of an HSV-CNS infection, this can be achieved with the help of the two species-specific gG1 and gG2 ELISA tests.

Limits: The level of pathogen-specific antibodies against the gG1 or gG2 epitopes in the CNS at the time when the CSF sample is taken may still (or already) be too low to increase the AI. Therefore, if the gG1 or gG2 test is performed alone this may give a false negative result in some cases or in specific cases, meaning that the antibody index is neither not calculable, or is normal.

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents (IgG and IgM Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months

Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The Sekisui Virotech System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.

4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 Virotech ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- and IgM-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Sekisui Virotech recommends the following procedure:

1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgM-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) Virotech Units (VE)

The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE_{(\text{positive control})} = \frac{OD_{(\text{positive control})}}{OD_{(\text{cut-off control})}} \times 10$$

$$VE_{(\text{patient serum})} = \frac{OD_{(\text{patient serum})}}{OD_{(\text{cut-off control})}} \times 10$$

9.3 Interpretation Scheme IgG and IgM

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
4. In case of a positive IgM result a review of the result by observing the course of the IgG titer is recommended.

9.4 Limits of the Test

6. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
7. Despite all advantages of the gG2-assay there exist also notes towards the limits of the test: On one hand the therapy with Acyclovirdie may influence the antibody development (3) and on the other hand, the genetic variability of the gG2 protein may lead to gG2 negative HSV 2 strains.

10. Performance Data

10.1 Analytic sensitivity and specificity

HSV 1 (gG1)

325 sera have been tested in IgG and compared with an Immunoblot able to differenciate (gG1 and gG2 specific)

		gG1 HSV 1-specific ELISA Virotech	
n=325		negative	positive
Analytic Finding HSV 1 and HSV 2 Immunoblot IgG (able to differenciate)	HSV 1 negative	88	1
	HSV 1 positive	3	206

27 sera showing borderline results with the reference system or the ELISA have not been considered.

Thus, an analytic sensitivity of 98,6% and an analytic specificity of 98,9% have been calculated.

HSV 2 (gG2)

346 sera have been tested in IgG and compared with an Immunoblot able to differenciate (gG1 and gG2 specific)

n=346		gG2 HSV 2-specific ELISA Virotech	
		negative	positive
Analytic Finding HSV 1 and HSV 2 Immunoblot IgG (able to differenciate)	HSV 2 negative	199	2
	HSV 2 positive	3	124

18 sera showing borderline results with the reference system or the ELISA have not been considered.
Thus, an analytic sensitivity of 97,6% and an analytic specificity of 99,0% have been calculated.

10.2 Prevalence (expected values)

HSV 1 (gG1)

IgG

The following table shows the results obtained with the Sekisui Virotech ELISA for selected sera collectives. The epidemiological data described in the literature are listed in comparison.

Sera Collective	positive with the VT ELISA in %	Literature Statement
Blood donors (n= 120)	71,7%	80% in Germany (15) 75% in Germany (10) 80% in Switzerland (6)
Infant sera (n=39)	33,3%	30% : 1-5 year old 50% : 12-16 year old (12)
Prostitutes sera (n=39)	82,1%	-
Crossreactive sera (n=39)	51,3%	-
Pregnant women´s-sera (n=51)	72,5%	70% in Netherlands (13)

Potentially cross-reactive sera (EBV, VZV, Measles, Parvo, CMV) and sera of pregnant women do not show an increased percentage of positive sera compared to the blood donors. This confirms the excellent specificity of the assays.

HSV 1 (gG1)

IgM

The following table shows the results obtained with the Sekisui Virotech ELISA for selected sera collectives:

Sera Collective	Positive with the VT ELISA in %
Blood donors (n= 120)	0
Infant sera (n=40)	0
Prostitutes sera (n=40)	0
Pregnant women´s sera (n=52)	0
Crossreactive sera (n=40)	5

HSV 2 (gG2)

IgG

The following table shows the results obtained with the Sekisui Virotech ELISA for selected sera collectives. The epidemiological data described in the literature are listed in comparison.

Sera Collective	positive with the VT	Literature Statements

	ELISA in %	
Blood donors (n= 120)	7,5%	15% in Germany (15) 14-18% in Germany (10) 19% in Switzerland (6)
Infants sera (n=39)	0%	1-5 year old< 2% 6-11 year old < 3% 11-16 year old approx.8% (12)
Prostitutes sera (n=39)	69,2%	78% in Germany (10) 49% in Switzerland (8)
Crossreactive sera (n=39)	7,7%	-
Pregnant women´s sera (n=51)	5,9%	-

Potentially cross-reactive sera (EBV, VZV, Measles, Parvo, CMV) show an identical percentage of positive sera compared to blood donors sera. Sera of pregnant women show a lower percentage of positive sera compared to blood donors sera. This confirms the excellent specificity of the assays.

HSV 2 (gG2)

IgM

The following table shows the results obtained with the Sekisui Virotech ELISA for selected sera collectives.

Sera Collective	Positive with the VT ELISA
Blood donors (n= 120)	1,7 %
Infants sera (n=40)	0 %
Prostitutes sera (n=40)	0 %
Pregnant women´s sera (n=52)	0 %
Crossreactive sera (n=40)	0 %

10.3 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested in a chessboard pattern with two sera. The obtained coefficients of variation for IgG for HSV 1 (gG1) and HSV 2 (gG2) are < 15%.

10.4 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 10 independent test runs on three different testdays. The obtained variation coefficient values for HSV 1 (gG1) and HSV 2 (gG2) are <15%.

11. Literature

1. Anzivino, E, D Fioriti, M Mischitelli, A Bellizzi, V Barucca, F Chiarini, V Pietropaolo. 2009. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. Virol J. 6,40
2. Arvin, A, C Prober. 1995. Herpes Simplex Viruses. 876-883. In Murray, P, E Baron, M Pfaller, F Tenover, and R Yolkenet (eds.). Manual of Clinical Microbiology. 6th Ed. ASM, Washington, D.C.
3. Bernstein, DI, LR Stanberry, CJ Harrison, JC Kappes, MG Myers. 1986. Antibody response, recurrence patterns and subsequent herpes simplex virus type 2 (HSV-2) re-infection following initial HSV-2 infection of guinea-pigs: effects of acyclovir. J Gen Virol. 67, 1601-1612
4. Brown ZA, Benedetti J, Ashley R, Burchett S, Selke S, Berry S, Vontver LA, Corey L.. 1991.Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. N Engl J Med. 324, 1247-1252
5. Brown, Z , S Sleke, J Zeh, J Kopelmann, A Maslow , R Ashley, D Watts, S Berry, M Herd, L Correy. 1997. The acquisition of herpes simplex virus during pregnancy. N Engl J Med. 337, 509-515
6. Bünzli, D, Wietlisbach V, Barazzoni F, Sahli R, Meylan PR. 2004. Seroepidemiology of Herpes Simplex virus type 1 and 2 in Western and Southern Switzerland in adults aged 25–74 in 1992–93 : a population-based study. BMC Infect Dis. 4. http://www.biomedcentral.com/1471-2334/4/10
7. CDC. 1998. Guidelines for Treatment of Sexually Transmitted Diseases. MMWR. 47, 1-118

8. Eing, BR Lippelt L, Lorentzen EU, Hafezi W, Schlumberger W, Steinhagen K, Kühn JE. 2002. Evaluation of confirmatory strategies for detection of type-specific antibodies against herpes simplex virus type 2. J Clin Microbiol. 40, 407-413.
9. Prober, C, W Sullender, L Yasukawa, D Au, A Yaeger, A Arvin. 1987. Low risk if herpes simplex virus infections in neonates exposed to the virus at the time of vaginal delivery to mothers with recurrent herpes simplex virus infections. N Engl J Med. 316, 240-244
10. Rabenau HF, Buxbaum S, Preiser W, Weber B, Doerr HW. 2002. Seroprevalence of herpes simplex virus types 1 and type 2 in the Frankfurt am Main area, Germany. Med Microbiol Immunol. 190, 153-160
11. Roizman, B, DM Knipe. 2001. Herpes Simplex Viruses and Their Replication. . In Fields, B, D Knipe, P Howley, et al. (eds.). Fields Virology 4th Ed. Lippincott-Raven, Philadelphia
12. Smith J, N Robinson. 2002, Age-specific prevalence of infection with herpes simplex : a global review . J Inf Dis. 186, 3-28
13. Tunbäck, P, Bergström T, Claesson BA, Carlsson RM, Löwhagen GB. 2007. Early acquisition of herpes simplex virus type 1 antibodies in children-A longitudinal serological study. J Clin Vir. 40, 26-30
14. Whitley, R. 2001. Herpes Simplex Viruses. In Fields, B, D Knipe, P Howley, et al. (eds.). Fields Virology 4th Ed. Lippincott-Raven, Philadelphia
15. Wutzler, P, Doerr HW, Färber I, Eichhorn U, Helbig B, Sauerbrei A, Brandstädt A, Rabenau HF. 2000. Seroprevalence of herpes simplex virus type 1 and type 2 in selected German populations - relevance for the incidence of genital herpes. J Med Virol. 61, 201-207

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **IgG-Samples – Dilution 1:101**

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

▼ **IgM-Samples - Dilution 1:101**
Rheumafactor-absorption with RF-SorboTech

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG, IgM
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**